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Full Length Research Paper

Using genomics to gain insights into the evolution and biology of *Pseudomonas syringae* pv. *aesculi* on European horse chestnut

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***Pseudomonas syringae* pv. *aesculi* (*Pae*) causes a devastating bleeding canker disease of European horse chestnut (*Aesculus hippocastanum*) in northwest Europe. The pathogen can enter woody branches directly via lenticels, leaf scars and nodes, causing lesions in the cortex and phloem. Cankers can expand rapidly, causing bleeding symptoms on the stem and branches, and the trees suffer progressive crown dieback often leading to mortality. To gain insights into the evolutionary and biological adaptations of *Pae*, the draft genome sequences were generated for four strains of *Pae* including three strains from Britain and the type strain from India that causes leaf spot on Indian horse chestnut (*Aesculus indica*). The genomic data suggest that the British and Indian *Pae* strains share a recent common ancestor and that the three British *Pae* strains descend from a single, very recent introduction of the bacterium into Britain. A phylogenetic analysis based on a set of conserved genes showed that *Pae* belongs to a distinct clade of *P. syringae* pathovars adapted to woody hosts. Genomic comparisons with other *P. syringae* pathovars showed that *Pae* has acquired genes that may enable it to infect and live within the woody parts of the tree. These include genes involved in the degradation of plant-derived aromatic compounds, and others which likely have a role in disabling the tree's defense responses. These genes have not yet been found in other pathovars of *P. syringae* that infect herbaceous plants but may be conserved in other tree-infecting bacteria and thus, may be important to our understanding of the infection processes of bacterial tree diseases.**

Key words: Bleeding canker, *Aesculus hippocastanum*.

INTRODUCTION

There has been an unprecedented recent increase in the numbers of hitherto unrecognised diseases attacking trees throughout the world. Many of the causal organisms have been inadvertently introduced into new ecosystems through the increase in global commerce, via pathways such as trade in live plants, including soils (Brasier, 2008). Bleeding canker of European horse chestnut (*Aesculus hippocastanum*) is a destructive new disease affecting hundreds of thousands of European horse chestnut trees across several countries in northwest Europe, resulting in severe damage to rural and urban amenity landscapes (Webber et al., 2008; Green et al., 2009). Disease symptoms include bleeding cankers located on the stem and branches, foliar discoloration, and crown dieback often leading to tree death (Green et al., 2009). The causal agent was identified as the gram-negative fluorescent bacterium, *Pseudomonas syringae* pathovar *aesculi* (*Pae*) based on a partial sequence for its gyrase B gene, which was identical to that of the *Pae* type strain isolated from leaf lesions on Indian horse chestnut (*A. indica*) in India (Durgapal, 1971; Durgapal and Singh, 1980). Prior to the European epidemic, this was the only location where *Pae* had been reported. This

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suggests that *Pae* may have originated from India and been recently introduced into Europe.

Pae is highly virulent on European horse chestnut and apparently very mobile, since the pathogen can spread rapidly between, and within, infected trees causing dieback and mortality when it kills a large proportion of the phloem in the branches and stem. Observations of naturally infected trees have provided strong evidence that *Pae* initiates infection of European horse chestnut via lenticels, leaf scars and other natural openings in branches of various ages (Steele et al., 2010). *Pae* then colonises the cortex, phloem and cambium and has the potential to form extensive, continuous cankers within a single growing season. Thus, part of the success of *Pae* as a tree pathogen and the causal agent of a large-scale epidemic is due to an apparently highly effective capacity for direct aerial infection and colonisation of the woody parts of its host (Steele et al., 2010). Very little is known about the virulence traits of *Pae*. Due to its aggressiveness and rapidity of spread, *Pae* presents an excellent model system for gaining a greater understanding of bacterial tree diseases (Green et al., 2010).

Good quality draft genome sequences were generated for a strain of *Pae* recently isolated from a diseased European horse chestnut in Britain (strain 2250) as well as the Indian type strain of *Pae* (Green et al., 2010). Whole-genome re-sequencing data were also generated for two additional *Pae* strains (P6617 and P6623) from different geographical locations in Britain (Green et al., 2010). The aim of this study was to gain insights into the biology and evolution of *Pae* strains causing the current disease epidemic on European horse chestnut by comparing the *Pae* genome with sequences from other *P. syringae* pathovars and by determining the genomic variation among all four *Pae* strains.

MATERIALS AND METHODS

Pae strain 2250 was isolated in 2008 from necrotic phloem in the stem of a diseased horse chestnut near Pitlochry, Perthshire, Scotland. *Pae* strains P6617 and P6623 were isolated in 2006 from diseased horse chestnuts in Glasgow, Scotland and Farnham, England, respectively. Prior to sequencing, the pathogenicity of strain 2250 was confirmed by inoculating a cell suspension on to wounded horse chestnut shoots and observing subsequent development of lesions. The Indian strain of *Pae* (NCPPB3681) was isolated in 1969 from a leaf lesion on Indian horse chestnut in Northern India (Durgapal, 1971). DNA preparation and sequencing, genome assembly and alignment, bioinfomatic analyses and phylogenetic analyses were carried out as described in Green et al. (2010).

RESULTS AND DISCUSSION

Genome-wide Illumina sequence data were generated for the three British *Pae* strains and the Indian type strain of *Pae.* Since *Pae* is a recent disease of unknown origin, it is important to confirm the taxonomic placement of the British *Pae* strains and determine the evolutionary relationships between *Pae* and other *P. syringae* pathovars. On the basis of seven house-keeping genes (Sarkar and Guttman, 2004) the British *Pae* strains were identical to the Indian *Pae* type-strain. The close phylogenetic relationship of the British and Indian *Pae* strains is consistent with their classification within the same pathovar of *P. syringae* (Green et al., 2010). The nucleotide sequences of these seven marker genes were also used in phylogenetic analyses which showed that *Pae* belongs to a distinct clade of *P. syringae* pathovars adapted to woody hosts. These niche changes are likely to have required host-specific genetic adaptations (Green et al., 2010).

Several economically important tree diseases are caused by *P. syringae*, including pvs. *syringae* and *morsprunorum* on stone fruit, *savastanoi* on olive and *avellanae* on hazelnut, but their virulence traits remain unknown (Kennelly et al., 2007). In this study, comparative genomic analyses revealed genomic regions in *Pae* which are absent from other *P. syringae* pathovars that infect herbaceous hosts and which represent candidate genetic adaptations to infection of the woody parts of the tree. Of particular significance are the pathways for the degradation of plant-derived aromatic compounds such as lignin derivatives and other phenolics (Green et al., 2010). It is possible that these pathways enable *Pae* to utilize as carbon sources aromatic substrates specifically derived from the tissues of woody plants. Another mechanism in *Pae* that might be important to survival during host infection is the presence of two genes that have a predicted function in nitric oxide metabolism. Both enzymes encoded by these genes have a role in the protection of bacteria from NO which is an antimicrobial toxin shown to play a key role in plant disease resistance (Delledonne et al., 1998). The *Pae*-specific pathways identified here are potentially highly important for the understanding of bacterial diseases of woody plants (Green et al., 2010) and further studies are required to elucidate their function.

Rates of single nucleotide polymorphisms in the four *Pae* genomes indicated that the three British *Pae* strains diverged from each other much more recently than they diverged from the Indian strain of *Pae*. The lack of genetic diversity among the three geographically distinct *Pae* strains from Britain (only one or two nucleotide differences across 3 M bp) is consistent with a single introduction of the pathogen within the last few years (Green et al., 2010). This serves to highlight the environmental risks posed by the spread of exotic plant pathogens into new geographical locations.

Conclusions

Pae strains on European horse chestnut share a common ancestor with a *Pae* type strain isolated from Indian horse chestnut in India. The data also indicate that the

three British strains descend from a single, very recent introduction of the bacterium into Britain. Genomic comparisons with other *P. syringae* pathovars show that *Pae* has acquired genes that may enable it to infect and live within woody tree tissues. These genes have not yet been found in other pathovars of *P. syringae* that infect herbaceous plants but may be conserved in other tree-infecting bacteria and thus, may be important to our understanding of the infection processes of bacterial tree diseases.

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